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Systemic and Cellular Reflections on Ageing and the Circadian Oscillator – A Mini-Review

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Abstract
From circulation to digestion to excretion, a circadian clock synchronizes most aspects of mammalian physiology with the solar day. During normal ageing, this daily coordination gradually erodes, and during pathological ageing such erosion is exacerbated. Recent experiments suggest that therapies aimed at sustaining circadian function increase quality of life in elderly patients. Hence, a better understanding of the interactions between the circadian clock and ageing – at both cellular and systemic levels – could lead to direct benefits for aged individuals.

From archaebacteria to humans, a biological clock has governed most aspects of cellular and systemic function. In mammals, 10% of all genes are expressed rhythmically in daily fashion, coordinated with the solar day. Hence, it is not surprising that nearly all aspects of physiology and behavior are governed by this 'circadian' oscillator [1]. Within the circulatory system, heartbeat and blood pressure vary in diurnal fashion, and rhythmic expression of genes involved in fibrinolysis are believed to contribute to the prevalence of infarctus at morning hours [2]. Similarly, circadian detoxication of many xenobiotic substances is controlled by cyclic expression of cytochrome enzymes [3], and daily variations in mood and alertness might be explained in part by circadian expression of ion channels, neuronal receptors and hormones [4, 5].

As the body ages, this coordination deteriorates in universal fashion: the circadian organization of the sleep-wake cycle is disrupted, and with it most other physiological manifestations of the circadian clock become less pronounced [6]. In situations of pathological ageing such as dementia as well as Huntington's and Parkinson's diseases, initial disease stages are marked by abnormal daily behavior, and later stages are correlated with almost total loss of circadian function [7, 8]. An understanding of this deterioration could therefore be of great assistance in increasing the quality of life for elderly individuals. Incorrectly, many of these disturbances are ascribed simply to 'sleep difficulties': in Western countries, 1 in 5 elderly people reports taking a sleep medication regularly [9].

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While these may provide a momentary alleviation of fatigue, underlying causes remain unaddressed, leading to a chronic dependence upon these substances.

How Is Circadian Function Regulated?

In all mammals including humans, the suprachiasmatic nucleus (SCN) of the brain hypothalamus acts as a central clock tissue, coordinating all aspects of overt daily physiology and behavior. However, its mechanism is cell autonomous: each individual neuron of the SCN possesses a separate functional oscillator at the cellular level [10]. The individual clocks in each neuron are kept in phase with one another by intercellular communication involving neuropeptidergic signaling and electrical synapses [11, 12]. The nucleus as a whole then synchronizes circadian timing throughout the body, probably through a combination of diffusible factors and direct connections to other brain regions [13, 14]. In fact, 'slave' oscillators of similar or identical molecular mechanism to those in the SCN are present throughout the body as well. Dissociated cells of peripheral tissues such as fibroblasts appear to be able to keep time as accurately as SCN neurons in culture [15]. Regulation of daily physiology and behavior is probably a product of systemic regulation of some processes directly by the SCN, and indirect regulation of others directly by cellular clocks in other tissues [16]. Such a system has the potential benefit of temporary uncoupling between SCN and peripheral clocks. For example, if rodents are fed consistently at an abnormal time, their peripheral clocks in the liver, kidney and other organs will change their circadian phase to reflect the new feeding time, but the SCN will continue to remain in synchrony with the solar day [17, 18].

At a cellular level, the mammalian circadian oscillator is composed of interlocking feedback loops of transcription and translation. A variety of dedicated genes are used for this purpose: the products of the *Clock* and *Bmal1* loci activate transcription of a set of afternoon-active genes including the period and cryptochrome family via *cis*-acting E-box elements present in the promoter regions of these genes. PER and CRY proteins then oligomerize to form a repressive complex that counteracts CLOCK:BMAL1 activation and shuts off their own transcription so that the cycle can begin anew. Added robustness and control of this mechanism is provided by secondary feedback loops of transcription factors (for example the nuclear hormone receptors REV-ERBα and ROR-α), by phosphorylation (for example casein kinases 1 and 2, GSK3, and likely multiple others), and by chromatin-modifying factors, RNA-binding proteins and cellular metabolic components like cAMP [19]. Elegantly, because this clockwork is present in most cells of the body, it is also one way that is used to control circadian output directly: the same elements critical for transcription of clock genes are also present in the promoters of many clock-controlled genes, where they drive circadian transcription using proteins from the clock itself [20].

Entrainment

To better understand how circadian synchrony might be impaired in older individuals, it is important to understand how it is entrained in the first place. In the absence of external signals that entrain the clock, the mammalian circadian clock will 'free run' at its endogenous genetically programmed circadian period. This period is approximately but not exactly 24 h. In fact, that is why this biological clock is called 'circadian' – from the Latin 'circa diem' or 'around a day'. Thus, without external timing cues, an organism will eventually drift out of synchrony with the actual solar day. Preventing this are entrainment signals that come from the environment. In mammals, entrainment of the central clock in the SCN is a predominantly ocular process. Environmental light is transduced from the retina via both conventional rod and cone photoreceptors containing the photopigment rhodopsin, and via a special class of retinal ganglion cells containing the pigment melanopsin [21]. These cells project directly to the SCN. Without these retinal cells – for example, in some totally blind or enucleated individuals – the circadian clock free runs, leaving affected people with chronic jetlag-like symptoms [22].

Light transduction through the lens of the eye is reduced in elderly individuals, particularly in the short wavelength range (<480 nm) [23]. Elderly mice show much smaller light-induced clock changes ('phase shifting') than young ones [24], and elderly individuals attenuated phase advances by ocular bright light exposure in the morning [25]. In addition, there is a loss of retinal ganglion cells that transmit light signals to the circadian timing system in older animals of multiple species [26]. Hence, it has been speculated that one cause of circadian disturbances in elderly subjects is an inability to entrain properly to the environment. Such problems might be particularly acute in a clinical setting, where lighting can be irregular or even constant (for example, the hospital intensive care unit), or during nursing care when patients

receive little natural light. Recent human studies provide evidence for this hypothesis and show that the human circadian system is less sensitive to light in the elderly [27, 28]. Moreover, practically speaking, bright diurnal lighting in nursing homes has improved behavioral circadian rhythms in residents and improved sleep quality [29] as well as cognitive performance and mood [30]. Similar results are seen for demented individuals [31]. By contrast, constant bright lighting might also have deleterious effects: at least in animal models, constant light can desynchronize SCN neurons [32] and, as discussed at the end of this review, impaired clockwork is linked to a variety of pathologies including cancer and immune dysfunction.

The degree to which brain- and eye-related entrainment changes in the elderly are mirrored or relayed in peripheral tissues remains controversial: age-dependent changes in clock gene amplitude (such as *Per2* and *Per3* genes) can be observed in blood leukocytes and oral keratinocyes, but no age-dependent differences are seen in phase shifting in these cells [33, 34]. This question of entrainment of peripheral organs is discussed more fully next. It is clear, though, that relatively inexpensive and easy benefits can be obtained by ensuring that elderly patients under clinical care receive adequate light in circadian fashion.

Systemic Considerations

As mentioned already, the SCN 'master clock' is entrained by light, and it in turn entrains slave oscillators in most cells of the body via a myriad of redundant cues. One important class is nervous signals. The neurons of the SCN demonstrate spontaneous firing patterns in daily fashion [10, 35], and project to many other brain nuclei [13], where they are presumed to be important for sleep-wake cycles and cognitive function. Paradoxically, however, the first line of communication from the SCN to centers controlling locomotor activity is probably hormonal: animal experiments show that implantation of SCN neurons encapsulated in porous plastic are still capable of rescuing rhythmicity in SCN-lesioned animals [14]. In peripheral tissues, the sympathetic nervous system also plays an important role, and has been shown to communicate timing signals directly to the adrenal gland and other tissues [36]. This signaling methodology is likely one of the methods by which daily rhythms of corticosterone synthesis are generated [37].

A second class of signals is indirect products of the regulation of other brain centers by the SCN. Body temperature is one important class: even though daily body temperature varies by only 1–4°C in mammals, these faint daily fluctuations – probably controlled by SCN innervation of the preoptic anterior hypothalamus and by daily activity patterns – are sufficient to entrain peripheral tissues [38]. Similarly, daily patterns of feeding are likely cues for the entrainment of peripheral clocks in tissues throughout the body [17, 18], as well as a separate 'food-entrainable' brain oscillator that can control locomotor activity in the absence of the known circadian clock [39, 40]. In animals, alterations of either of these classes of signals effectively 'decouple' the central clock from oscillators in other tissues: either reversal of daily body temperature rhythms [38] or of daily feeding rhythms [17, 18] can inverse the timing of local clocks in peripheral organs. The exact mechanism of this regulation remains unknown, but recent research proposes direct molecular coupling between metabolic cycles and the circadian oscillator via redox-regulated 'sirtuin' proteins [41, 42], and such a metabolic link could be a plausible signal, at least for food-based entrainment. In reverse, brain-regulated corticosterone rhythms probably serve as a stabilizing influence: in animals lacking the glucocorticoid receptor in the liver, liver clocks are much more rapidly shifted by alterations in daily feeding patterns [43].

From these experiments, one can speculate that another way in which circadian oscillations might be dampened in elderly individuals might be through alterations of systemic entrainment pathways. For example, in one study, aged rats showed normal entrainment of the SCN by light, but severely disrupted liver clock entrainment [44]. We hypothesize that well-characterized alterations in the hypothalamic-adrenal-pituitary axis in elderly individuals might play a major role. For example, circadian oscillation of cortisol is dampened, peak levels are reduced and evening levels are increased in older people [45]. This condition would act synergistically: not only would the direct circadian effects of cortisol upon digestion and detoxification be lost, but also any effects of irregular feeding and body temperature would have even greater effects upon the circadian oscillator because of impairment of parallel cortisol-based entrainment mechanisms. Multiple studies have shown that imposed regular routines of mealtime and exercise improve circadian consolidation of sleep-wake cycles in elderly individuals [29, 46]. Physiologically speaking, meal and light routines might help substitute for the loss of internal circadian

signals, and themselves act as timing cues. Under normal conditions in young, healthy individuals, factors such as meal timing are overpowered by systemic circadian cues, and therefore might play a less important role. A similar situation exists in blind individuals: in the absence of the strong influence of ocular light, many subjects can be entrained to the 24-hour day by weak nonphotic timing cues that do not play a significant role for sighted individuals [47].

Cellular Considerations

As already described, virtually all cells in mammals have an independent circadian clock capable of sustained oscillations in isolation. Because each cellular oscillator is slightly different, though, circadian oscillations in dispersed cultures or tissue slices of peripheral cells rapidly dampen. The function of the redundant signaling described above is to synchronize all cellular clocks, thereby sustaining robust rhythms of gene expression and physiology at the tissue level. Recent studies suggest that there is a second function, though: to drive a subset of cellular circadian gene expression directly. Thus, genetically modified mice lacking a functional circadian clock in a particular organ lose circadian expression of a subset of genes (those coupled to the cellular oscillator) but still show circadian expression of others (those coupled to systemic mechanisms) [48, 49]. Similarly, tissue-specific rescue of a clock gene results in rescue of only a subset of physiological aspects of the underlying mutant strain [50].

The cellular heterogeneity of clock properties probably serves a definite function. Although the day length on our planet is fixed to 24 h, the period of light during this time can vary dramatically in lateral clines. To cope with these differences, the circadian clock is able to track both light onset (dawn) and light offset (dusk). Because of cellular differences in the SCN, some of its cellular clocks are phased earlier, and thereby track dawn, and others are phased later to track dusk – a hypothesis formed long before the existence of cellular clocks was discovered [51], and recently confirmed in rodent SCN neural activity studies [52] as well as luciferase imaging experiments [53]. To keep SCN cells locally synchronized, neurochemical coupling mechanisms are used [12, 54], and probably gap junctions as well [55]. Elimination of these components in mice results in dramatically dampened circadian oscillations.

These cellular mechanisms comprise yet another way in which circadian rhythms could be altered in older individuals. It is possible that cellular clocks are themselves altered in aged cells, either intrinsically or due to increased release of inflammatory cytokines which are known to affect circadian gene expression [56, 57]. Alternatively, changes in synaptic patterns and neurochemical coupling within the SCN could also result in alterations in cellular clock function [58, 59]. Lending credence to this hypothesis, SCN electrical firing shows reduced circadian amplitude in older rats [60, 61] and reduced sensitivity to the hormone melatonin [62]. Transplantation of a fetal SCN into aged hamsters improved behavioral rhythmicity [63, 64].

Circadian Alterations in the Elderly: Working Backwards

A great deal of research has been done to characterize the specific physiological changes that occur as humans age, and some of these have already been mentioned above. The nature of these changes could permit us to understand better which of the mechanisms presented above might be responsible for the decline in circadian function in elderly individuals.

Even based upon simple questionnaires, it is clear that from adolescence onward, human circadian clocks move earlier as individuals age. Between the ages of 20 and 80 years, the timing of sleep shifts an average of 2 h [65]. At the same time, sleep consolidation – that is the ability to sleep in long, unbroken nighttime bouts – is decreased. In addition, the total amount of slow-wave 'deep' sleep decreases and rapid-eye-movement 'dream' sleep is more evenly distributed [66]. In short, there is a marked decrease in the 'circadian amplitude' of sleep. Sleep patterns are believed to be the sum of two independent processes: a circadian one that programs human beings to sleep preferentially at night and a homeostatic one that increases sleep pressure with increasing time awake [67]. In principle, the fragmentation of sleep could be caused either by a reduction in the strength of the circadian component or by a change in the homeostatic component, and this question is actively debated at present.

The reduction in circadian amplitude is not limited to sleep: it is universal and results in a variety of endocrine consequences. Appetite is reduced during meals and intermeal eating increases, and activity is increased during nighttime hours, especially during pathological ageing (dementia, Huntington's and Parkinson's disease). Mean-

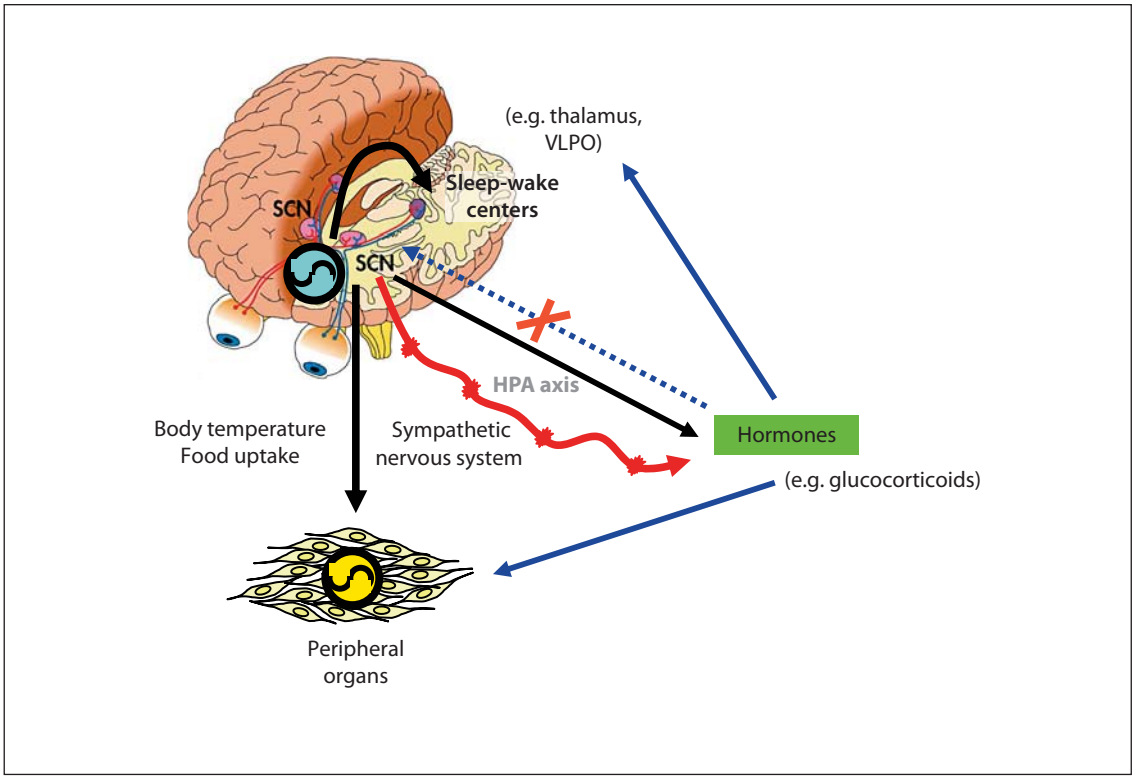

Fig. 1. Interaction between master and peripheral clocks. In mammals, the master clock in the SCN communicates timing information to other tissues in the brain and body via a redundant combination of signals: nervous innervation of other brain regions, secreted hormones, and indirect cues like body temperature and food intake (shown as black arrows leading out from the SCN), as well as by sympathetic innervation of peripheral organs (red arrow). Hormonal signals that influence circadian phase can in turn affect both peripheral organs and sleep-wake centers (blue arrows). We suggest that some of these hormones (such as glucocorticoids) cannot signal to the SCN (dashed blue arrow), though they can still affect sleep-wake centers. In older individuals, the circadian amplitude of signals from the SCN is decreased, resulting in sleep fragmentation and reduced amplitudes of circadian behavior and physiology. We propose that at the same time, changes in hormonal balance – perhaps in the hypothalamic-pituitary-adrenal (HPA) axis – would additionally alter the circadian phase of behavior. Because these hormones do not 'feed back' to the SCN, they would not change circadian period length under free-running conditions.

while, the circadian amplitude and magnitude of many hormones are reduced, especially melatonin, prolactin, those of the hypothalamic-adrenal-pituitary axis as well as glucose [68]. Although some studies are contradictory on this point, body temperature may be both lower and oscillate with lower circadian amplitude [69]. Endothelial nitric oxide oscillations are also reduced and may be responsible for the damping of circadian cardiac function [70]. In short, it is easy to imagine how circadian physiology might be attenuated.

A thornier problem is what might shift phase. One obvious hypothesis to explain earlier phase would be a shortening of the period of the circadian oscillator. Intrinsic period shortening has been observed in aged rodents and nonhuman primates by some groups [71, 72]. In humans, laboratory studies make this hypothesis unlikely: cross-sectional studies of younger and older adults under 'forced desynchrony' – artificial lighting conditions that measure the intrinsic period of the circadian oscillator unaffected by external environment – show no differences in average period length [73]. Interestingly, the same studies also show that the timing ('phase angle') of sleep relative to the circadian clock (measured via the pattern of secretion of the circadian hormone melatonin) is earlier and sleep is less consolidated [74, 75]. It is this lack of sleep consolidation that has led other groups to speculate that sleep fragmentation is itself responsible for the circadian phase shift [76].

According to this idea, age-related changes in sleep structure and sleep consolidation reflect a reduction in

the circadian force that opposes homeostatic sleep pressure [77]. This influence is particularly significant in the late evening, when the circadian drive for wakefulness is highest (that is, the wake maintenance zone), and in the early morning hours, when the circadian drive for sleep is at its maximum, thus leading to more sleep episodes in the evening and earlier morning wakening in elderly individuals. As a consequence of this altered sleep-wake pattern, the elderly may expose themselves to more morning than evening light, which may result in an earlier circadian phase.

This model works well in normal conditions, but it does not fully explain similar results studying sleep latency under forced desynchrony conditions that look at the free-running circadian oscillator [73]. We suggest that this paradox might be explained by hormonal changes, and that the same hormonal differences might also explain the reduced circadian drive discussed previously. Since many hormones phase shift peripheral circadian clocks, alterations in their overall levels would be predicted to alter period in free-running conditions and phase in an entrained environment. However, if these hormones do not act upon the SCN – due to the blood-brain barrier or a lack of the appropriate receptors –, the result would be a shift in the phase of circadian physiology (controlled most directly by peripheral organs and brain regions) without a change in the free-running period of circadian behavior (controlled by the SCN) (fig. 1).

Pathological Effects of Reduced Circadian Function in Older Individuals

The obvious effect of decreased circadian amplitude is a decreased quality of life, probably driven by worse sleep and a feeling of less energy during active periods. An increasing number of mouse studies suggest that decreased circadian function might also have a direct effect upon life span. These studies fall into three classes. First, there are mouse studies showing that deletions of some clock genes (for example, *Bmal1* and *Per2*) lead to decreased life span and increased cancer as well as other age-related pathologies [78, 79]. Though interesting, metabolic defects linked to deletions of these genes make it unclear if their effects are gene or clock specific. Secondly, there are studies suggesting that circadian desynchrony per se – via chronic jet lag paradigms, for example – leads to decreased life span [80] and increased cancer rate and tumor growth [81]. Finally, an increasing number of mechanistic studies tie the circadian clock intimately to questions of metabolism. They show for example that the circadian clock times cell division in adult animals [82], and that it regulates and is regulated by proteins sensitive to cellular reduction and oxidation like sirtuins, which have themselves garnered a great deal of interest as anti-ageing proteins [83]. Thus, the circadian clock may be fundamentally tied to the balance of factors that prevent cellular damage, and reduced circadian amplitude may therefore accelerate this process.

Although not discussed herein, other pathological effects may also arise separately through the changes in sleep that come about during ageing. For example, sleep deprivation results in immune system dysfunction and chronic inflammation. Since these two effects are commonly observed in aged individuals who suffer from disturbed sleep, it is easy to hypothesize a causative link [84]. Other studies, however, suggest that, according to cognitive parameters, healthy subjects actually tolerate sleep deprivation *better* than younger ones, belying the idea that they are sleep-deprived because of their altered sleep structure [85]. Further research is clearly needed upon this interesting question.

Perspective on Experiments and Treatments

Because the synchronization of peripheral circadian oscillators can be driven by indirect cues such as mealtime and body temperature, some of the best current circadian therapies for elderly individuals may also be the simplest: timed regular activities and mealtimes as well as bright daytime light. Multiple reports from clinical settings already suggest that these measures can help. In the longer term, the susceptibility of peripheral clocks to multiple hormones may make hormonal therapies another viable option. Exactly which hormones might be effective – and which, if any, might be responsible for these changes in the first place – are experimentally testable hypotheses, particularly given easy access to peripheral clocks in some human tissues. Thus, it is likely that future experiments cannot only help illuminate the causes of circadian dysfunction in the elderly, but also help alleviate it in a safe manner.

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